

A Diagnostic Approach to Fungal Pneumonia

An Infectious Diseases Perspective



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Although bacteria significantly exceed fungi as the most common cause of lower respiratory tract infection, the incidence of fungal pneumonia is increasing because of a growing at-risk population of immunocompromised individuals as well as anthropogenic global heating and environmental disruption. When a patient presents with a clinical syndrome of pneumonia, a constellation of factors must be considered to determine the probability of a fungal pneumonia, including host factors, epidemiologic exposures, suggestive radiographic patterns, and the presence of a non-resolving pneumonia. In addition, knowledge of clinically important fungal pathogens, their epidemiology, and associated clinical syndromes are key in guiding appropriate diagnostic testing and result interpretation, and ultimately rendering a correct diagnosis of a fungal pneumonia. This article aims to provide a framework for the evaluation and appropriate diagnostic testing of patients with suspected fungal pneumonia.

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KEY WORDS: diagnostics; dimorphic; fungal infection; mold; pneumonia; pretest probability; yeast

Case Vignette

A 55-year-old woman with a history of kidney transplantation 4 years prior for end-stage kidney disease caused by diabetes, on maintenance immunosuppression with tacrolimus, mycophenolate mofetil, and low-dose prednisone presents with progressive nonproductive cough, fatigue, and low-grade fevers of 3 weeks' duration. She was treated for an episode of acute cellular rejection of her transplanted kidney 4 months previously with a pulsed regimen of high-dose steroids. She had gone on a 3-day hiking trip to Tapo Canyon Regional Park in Southern California 6 weeks earlier.

Vitals were notable for an oral temperature of 38.1 °C. Results of a physical examination were unremarkable.

Laboratory testing showed a low absolute lymphocyte count (0.6 cells/ μ L) and was otherwise normal. CT scan of the chest demonstrated multiple pulmonary nodules, including a 3-cm left upper lobe nodule. Induced sputum submitted for bacterial, fungal, nocardial, and mycobacterial staining and cultures remained negative at day 3 of hospitalization. A serum galactomannan antigen test was negative, and a serum (1 $\rightarrow$ 3)- β -D-Glucan test was positive at 297 pg/mL. She was initiated on IV piperacillin-tazobactam but did not have

ABBREVIATIONS: NGS = next-generation sequencing; PCP = *Pneumocystis jirovecii* pneumonia; PCR = polymerase chain reaction

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any appreciable clinical improvement by the third day of hospitalization.

This case highlights a constellation of factors that must be considered when assessing patients for the presence of a fungal pneumonia, including (1) host factors, (2) epidemiologic exposures, (3) suggestive radiographic patterns, and (4) nonresolving pneumonia without alternative infectious or noninfectious causes. In addition, knowledge of clinically important fungal pathogens, their epidemiology, and associated clinical syndromes are key in guiding appropriate diagnostic testing and result interpretation and in ultimately rendering a correct diagnosis of a fungal pneumonia.

Clinically Important Fungal Pathogens of Pneumonia

Up to 5.1 million species of fungi are in existence but only approximately 300 species are known to be pathogenic in humans.¹ These clinically important fungi can be grouped into three general categories in accordance with their morphology (Fig 1), a classification that is useful for workup within the clinical

microbiology laboratory as well as for clinical assessment. These include (1) unicellular yeast and yeast-like organisms, (2) multicellular molds that exist as mycelia (networks of hyphae), and (3) dimorphic fungi whose morphology depends on the ambient temperature (yeast at 37 °C; mold at 25 °C). Many fungi, especially dimorphics, are endemic to specific geographic regions, where they can be acquired by residents or travelers, whereas others such as *Aspergillus*, *Cryptococcus*, and *Pneumocystis* are ubiquitous. Inhalation of environmental fungal spores or arthroconidia by humans into terminal alveoli is a crucial part of the life cycle for many clinically important fungi (Fig 2), many of which primarily present with pneumonia.

When to Suspect a Fungal Pneumonia

Although bacteria significantly exceed fungi as the most common cause of lower respiratory tract infection,^{2,3} patients presenting with a syndrome of pneumonia must be further assessed for the presence of a fungal infection when certain host, epidemiologic, radiographic, and clinical factors are present. Each of these factors is discussed in detail below.

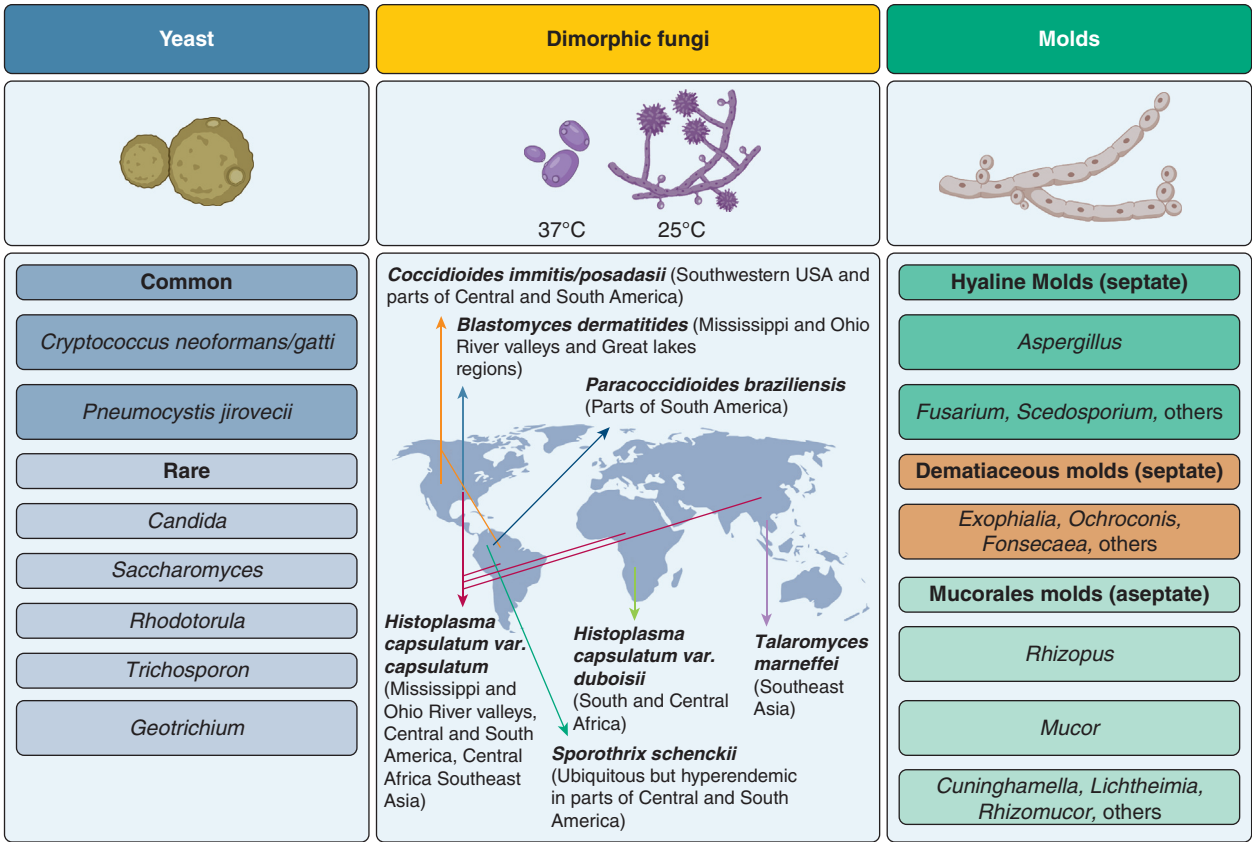


Figure 1 – Clinically important pathogens of fungal pneumonia.

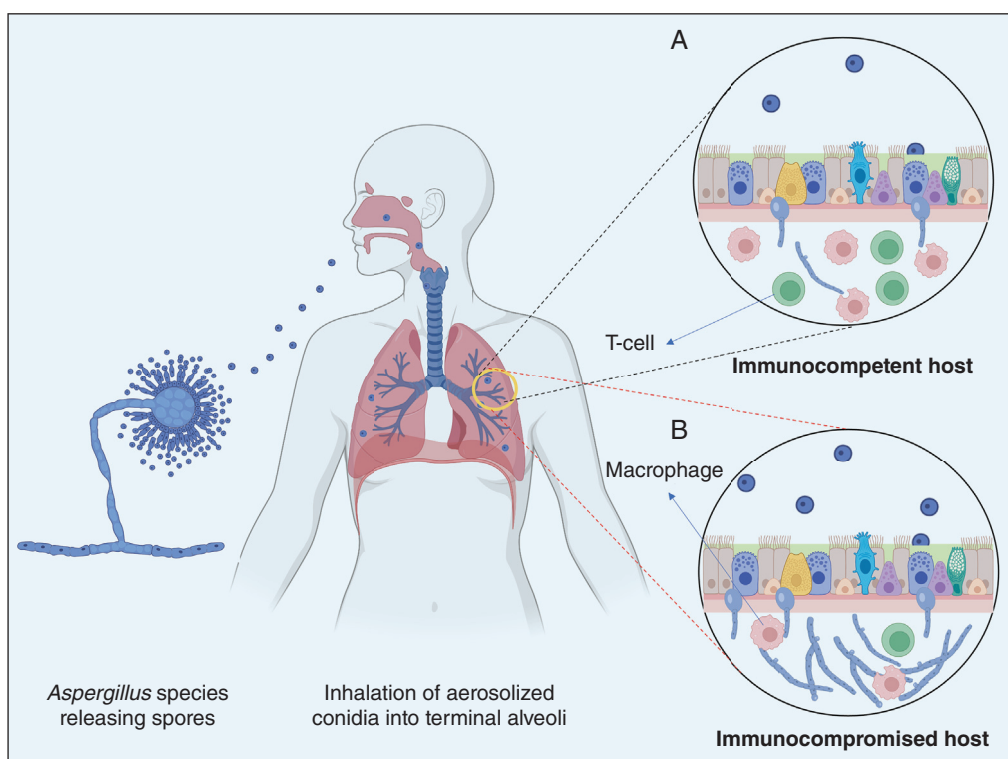


Figure 2 – Pathophysiology of fungal pneumonia. A, In the presence of a normal immune system, inhaled conidia are phagocytosed by alveolar macrophages and neutrophils, abrogating the process of fungal germination and dissemination. B, In the presence of neutropenia, T-cell lymphopenia or T-cell dysfunction, inhaled conidia (such as from *Aspergillus* spp) germinate into hyphae, followed by uncontrolled hyphal growth and dissemination. Invasive yeastlike *Cryptococcus* can similarly evade a weakened immune system and disseminate.

Host Factors

Immunocompromised Hosts: Most fungal pneumonias occur in individuals with prolonged and profound neutropenia or with compromised cellular immunity, such as people with HIV/AIDS, hematologic malignancies, bone marrow or organ transplantation, long-term corticosteroid therapy, and autoimmune conditions requiring immunosuppressive therapies (Table 1). CD4⁺ T-cells are particularly critical for control of fungal pathogens. Th1 CD4 cells secrete tumor necrosis factor alpha, granulocyte-macrophage colony-stimulating factors, and interferon-gamma, cytokines that recruit inflammatory cells to the site of infection and promote granuloma formation, thus abrogating fungal dissemination (Fig 3).⁴ Th17 cells secrete IL-7 and IL-22, leading to neutrophil recruitment with release of reactive oxygen species, neutrophil extracellular traps, and defensins to entrap and destroy fungi (Fig 3), especially within mucosal surfaces. The risk for infection with a particular fungal pathogen is not uniform across immunocompromised populations and depends on the presence and intensity of neutrophil and T-cell compromise, occurrence of co-morbidities (lung and liver disease in particular),

advanced age (leading to T-cell immunosenescence), the time elapsed from the immunocompromising event, and the use of prophylactic antimicrobials.⁵ Low absolute lymphocyte count is associated with increased risk for fungal and other opportunistic infections, including *Pneumocystis jirovecii* pneumonia (PCP) in recipients of transplants.^{6,7} Similarly, the incidence of fungal infections including pneumocystosis, cryptococcosis, and histoplasmosis increases in patients with HIV/AIDS with a CD4 count below 200 cells/ μ L.⁸

Hosts With Chronic Organ Dysfunction: Hosts with chronic organ dysfunction are at increased risk for infection with certain fungi. Chronic lung disease from conditions such as emphysema, bronchiectasis, sarcoidosis, or prior TB are associated with an increased risk for chronic pulmonary aspergillosis and aspergillomas as well as for chronic pulmonary histoplasmosis.^{9,10} Cirrhosis is a recognized risk factor for infection with *Cryptococcus neoformans*,¹¹ whereas patients with uncontrolled diabetes and diabetic ketoacidosis are uniquely susceptible to mucormycosis, particularly invasive sinusitis and pneumonia, because of

TABLE 1] Host Factors for Fungal Pathogens of Pneumonia

Fungal Pathogen	Host Factors
<i>Aspergillus</i>	• HSCT (especially allogenic with GVHD) • Hematologic malignancy with prolonged neutropenia (≥ 2 wk of an absolute neutrophil count of < 0.5) • SOT (especially lung and with recent treatment for organ rejection) • High-dose steroids (≥ 20 mg prednisone for ≥ 2 wk) • Ibrutinib (especially when combined with steroids)
Mucorales	• HSCT (especially allogenic with GVHD) • Hematologic malignancy with prolonged neutropenia (≥ 2 wk of an absolute neutrophil count of < 0.5) • SOT (especially lung, kidney, and with recent treatment for organ rejection) • High-dose steroids (≥ 20 mg prednisone for ≥ 2 wk) • Uncontrolled diabetes (risk of invasive sinusitis much higher than pneumonia)
<i>Pneumocystis jirovecii</i>	• HIV/AIDS with CD4 < 200 cell/mm³ (except if CD4 100-200 cell/mm³ and HIV viral load is suppressed) or CD4% $< 14\%$ • HSCT (especially allogenic with GVHD) • SOT (especially lung and with recent treatment for organ rejection) • High-dose steroids (≥ 20 mg prednisone for ≥ 2 wk) • Ibrutinib (especially when combined with steroids)
<i>Cryptococcus</i>	• HIV/AIDS with CD4 < 100 cell/mm³ • HSCT (especially allogenic with GVHD) • SOT (especially lung, liver, and with recent treatment for organ rejection) • High-dose steroids (≥ 20 mg prednisone for ≥ 2 wk)
Dimorphic fungi	• TNF-alpha inhibitors • High-dose steroids (≥ 20 mg prednisone for ≥ 2 wk) • HSCT (especially allogenic with GVHD) • SOT (especially lung, and with recent treatment for organ rejection) • HIV/AIDS with CD4 < 250 cell/mm³ (<i>Coccidioides</i>) • HIV/AIDS with CD4 < 150 cell/mm³ (<i>Histoplasma</i>) • HIV/AIDS with CD4 < 100 cell/mm³ (<i>Blastomyces</i>)

GVHD = graft-vs-host disease; HSCT = hematopoietic stem cell transplantation; SOT = solid organ transplantation.

elevated levels of free iron at an acidic pH that supports growth of Mucorales molds.¹²

Immunocompetent Hosts: Although most fungi require some degree of underlying immunocompromise or chronic disease to cause pneumonia, some organisms may lead to full-blown infection in immunocompetent hosts. Dimorphic fungal infections, including blastomycosis, coccidioidomycosis, histoplasmosis, paracoccidioidomycosis, sporotrichosis, and to a lesser extent talaromycosis are common in healthy hosts and may be severe if the infecting fungal inoculum is high. *Emergomyces canadensis*, an emerging dimorphic fungal pathogen, has been reported as a cause of pneumonia in healthy hosts.¹³ Infection with *Cryptococcus gattii* infection is frequently seen among immunocompetent patients, in which it may present as pulmonary cryptococcomas.¹⁴

Epidemiologic Factors

Certain epidemiologic risk factors increase the risk of fungal pneumonia. Antecedent severe infection with influenza¹⁵ or SARS-CoV-2¹⁶⁻¹⁸ is associated with an increased risk for invasive aspergillosis and

mucormycosis, and near drowning events are associated with *Scedosporium apiospermum* pneumonia.¹⁹ Individuals who inject drugs are at increased risk for candidemia with secondary hematogenous dissemination to the lungs (septic emboli), whereas *Candida* empyema is associated with extension from intraabdominal or intrathoracic sources, esophageal rupture, and pleural space manipulation.²⁰ Of note, primary *Candida* pneumonia is exceedingly rare in both immunocompetent and immunocompromised patients, accounting for only approximately 1% of proven cases of pneumonia in autopsy studies.²¹ Growth of *Candida* from respiratory cultures almost always represents colonization rather than infection. Cannabis and tobacco leaves may harbor several fungi, including *Aspergillus*, other molds, and *Cryptococcus*; smoking these products has been associated with an increased risk of pneumonia secondary to these organisms.^{22,23} Exposure to bird and bat guano, including within caves that contain high concentrations of fresh droppings, are risk factors for histoplasmosis and cryptococcosis. Several dimorphic fungi (*Histoplasma*, *Blastomyces*,

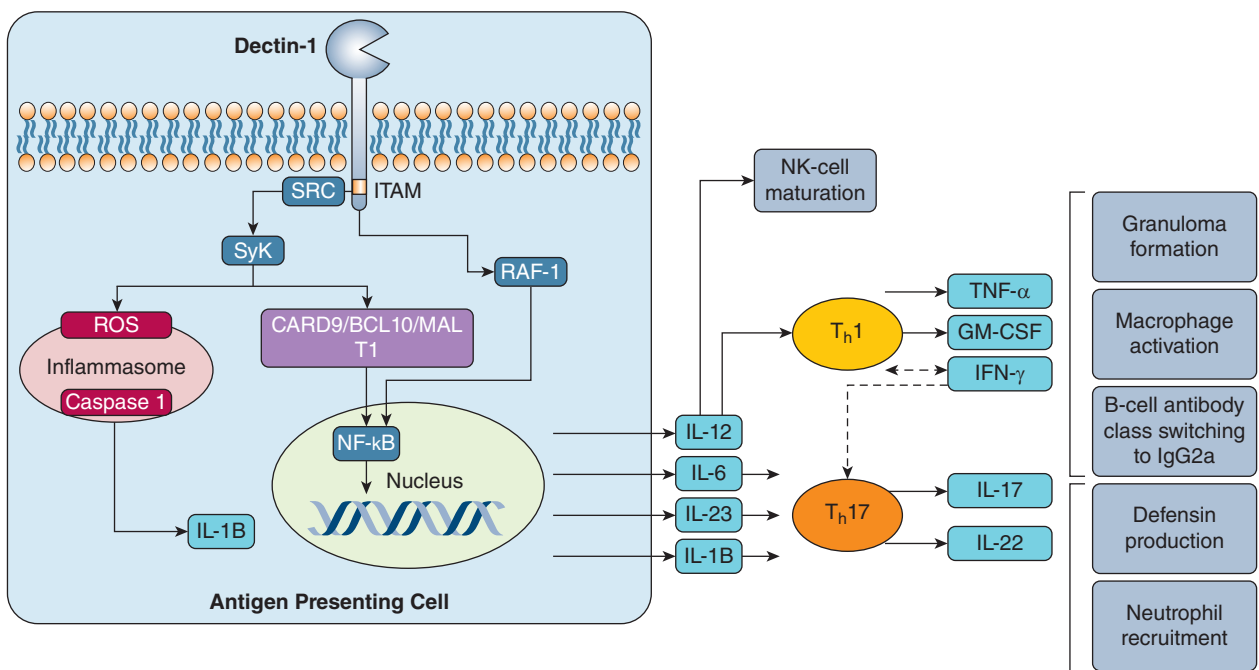


Figure 3 – The adaptive immune response to fungal infection. Binding of the (1→3)-β-D-Glucan fungal cell wall component and fungal pathogen-associated molecular pattern to the Dectin-1 human fungal pattern recognition receptor induces downstream activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a protein complex that controls DNA transcription and cytokine production as well as assembly of the nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3) inflammasome and production of reactive oxygen species. Reactive oxygen species damage invading fungal pathogens. The NF-κB complex and NLRP3 inflammasome promote secretion of pro-inflammatory cytokines including IL-1β, IL-6, IL-12, and IL-23. IL-1β and IL-23 activate T-helper 17 stimulate neutrophil recruitment to sites of infection and defensin production, which eradicates invading fungi. IL-12 induces Th1 cell differentiation then interferon-gamma release, which activates macrophages, promoting granuloma formation and fungal killing and encasement.

Coccidioides, *Paracoccidioides*, *Talaromyces*, and others) are endemic to certain geographic regions of the world and should be considered in residents of or travelers to or from those regions who present with pneumonia (Fig 1).

Suggestive Radiographic Patterns

Although radiographic findings of pneumonia on chest radiograph and CT scan are rarely pathognomonic for a particular cause, some patterns are suggestive of fungal infection. Fungal pneumonia typically affects the upper lobes, although any part of the lung can be involved.²⁴⁻²⁶ Radiographic manifestations vary, but pulmonary nodules are a common sign of fungal infections. There are several radiographic signs suggestive of angioinvasive mold. The presence of a nodule with surrounding ground-glass infiltrates, referred to as a halo sign (Fig 4A), and the “vessel occlusion sign,” a CT pulmonary angiography finding of arterial vessel interruption, may be indicators of invasive aspergillosis or, less commonly, other angioinvasive molds or nonfungal pathogens.^{27,28} The reverse halo sign, a rounded area of ground-glass opacities surrounded by a ring of consolidation, and the

bird’s nest sign (a reverse halo sign with irregular and intersecting areas of stranding) are most suggestive of angioinvasive mucormycosis, although other infectious (including invasive molds) and noninfectious (especially cryptogenic organizing pneumonia) origins are possible. Additionally, the presence of multiple pulmonary nodules, concomitant sinusitis, cavities, and pleural effusions increase the likelihood of mucormycosis.²⁹⁻³¹ Mediastinal lymphadenopathy raises the possibility of granulomatous fungal infections, particularly histoplasmosis and coccidioidomycosis (uncommon in blastomycosis).³² Diffuse, bilateral, interstitial infiltrates (Fig 4B), often with perihilar predominance, are characteristic of *Pneumocystis* pneumonia; less common radiographic features include pneumatoceles (Fig 4C), pneumothorax, and rarely, nodules.³³ Nonspecific peripheral cavitory lesions may be seen with septic emboli from *Candida* endocarditis (Fig 4D).

Nonresolving Pneumonia Without Alternative Cause

Pneumonias that progress or persist despite seemingly appropriate antibiotic or antiviral therapy should evoke

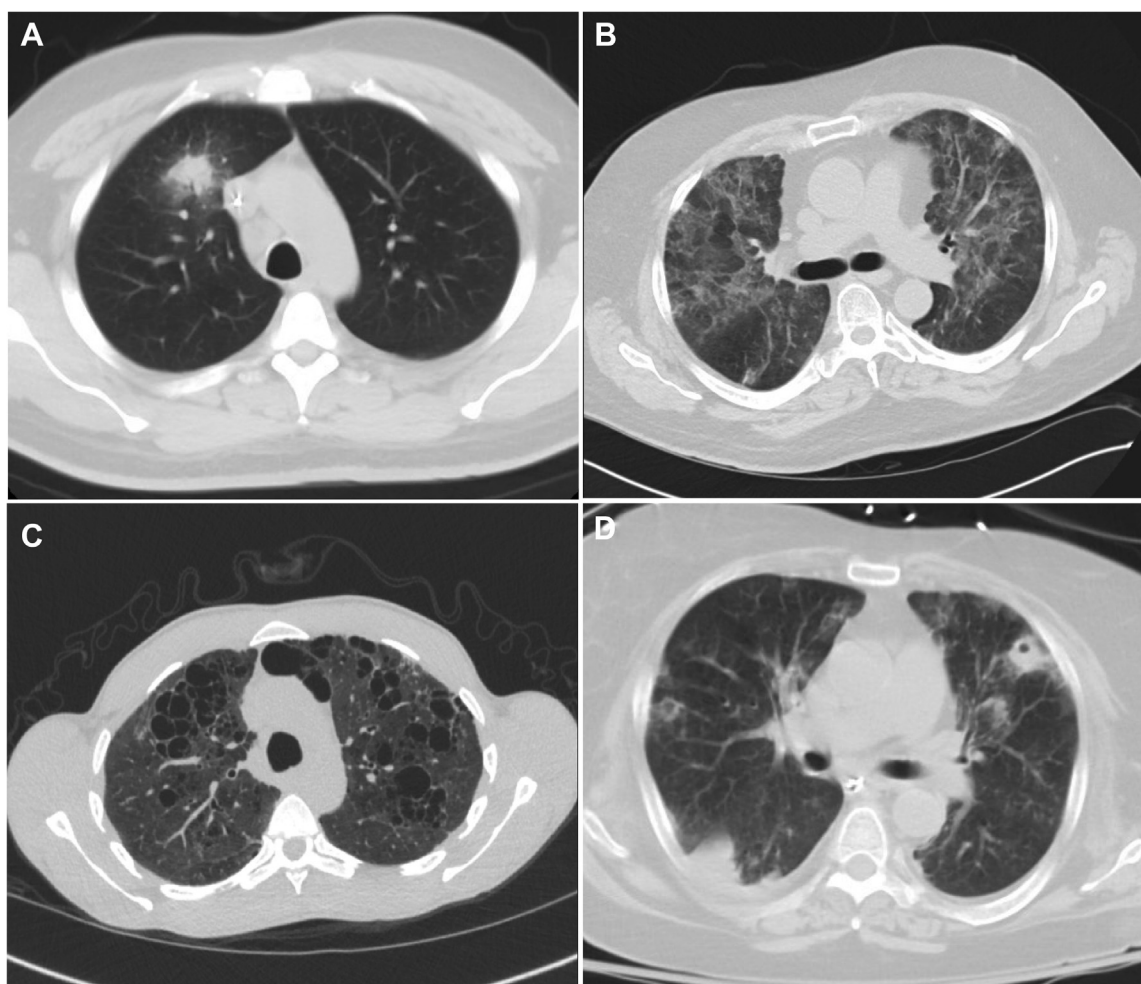


Figure 4 – A-D, Radiography of fungal pneumonia. A, Right upper lobe nodule with surrounding ground-glass opacities (halo sign) in a patient with invasive aspergillosis. B, Diffuse ground-glass opacities in a patient with *Pneumocystis pneumonia*. C, Diffuse ground-glass opacities and scattered thin-walled pneumatoceles in a patient with *Pneumocystis pneumonia*. D, Peripheral small cavitary nodules in a patient with *Candida endocarditis* and septic emboli to the lungs.

the possibility of an atypical process, including fungal pneumonia. *Coccidioidomycosis*, *histoplasmosis*, and *blastomycosis* are frequently misdiagnosed as community-acquired pneumonia even in endemic areas, leading to nonresolving pneumonia.³⁴⁻³⁶ Providers should consider these causes, especially in patients residing in or traveling through endemic areas. Negative respiratory and systemic diagnostic testing for bacterial infection reduce the posttest probability of bacterial pneumonia and increase the likelihood of alternative causes. Of note, the yield of bacterial testing, especially for culture-based methods, drops significantly with exposure to antecedent antibiotics.³⁷ Other atypical infections such as TB and *nocardiosis* should be considered. Noninfectious mimics of pneumonia such as *sarcoidosis*, malignancy, and cryptogenic organizing pneumonia also should be investigated. A nonresolving pneumonia without an alternative infectious or

noninfectious cause should prompt testing for fungal infection. In some cases, fungal pneumonia can present acutely, including with pulmonary mucormycosis, PCP in immunocompromised patients without HIV, and ARDS secondary to acute *histoplasmosis*, *blastomycosis*, *coccidioidomycosis*, and other endemic fungi.

Diagnostic Testing for Fungal Pneumonia

Once host, epidemiologic, radiographic, and clinical factors are taken into account, a pretest probability for fungal pneumonia can be established. A differential diagnosis for fungal infections (such as *aspergillosis*, *histoplasmosis*, or *pneumocystosis*) should be developed based on these same factors. When the pretest probability for a particular pathogen is moderate to high, diagnostic testing should be pursued. Testing can be more liberal in critically ill and immunocompromised

patients. Conventional tests for fungal infections include culture, microscopic examination, antigen testing, serology, and molecular testing (Table 2), performed on respiratory or nonrespiratory specimens; noninvasive testing with these modalities should be obtained first. If results of initial diagnostic testing are negative, invasive diagnostics, including bronchoscopy/BAL and tissue sampling through transbronchial or transthoracic biopsy, should be pursued and an expanded differential diagnosis should be considered. If testing remains negative but the suspicion for fungal infection remains high, advanced testing such as next-generation sequencing can be considered. Despite advances in fungal diagnostics, including peripheral biomarkers, sensitivity remains suboptimal, and tissue sampling remains the cornerstone of diagnosis. A diagnostic approach and algorithm for investigation of fungal pneumonias is proposed (Table 2, Fig 5).

Regardless of the specific test, high sample quality, especially for respiratory specimens (oral wash, expectorated/induced sputum, endotracheal fluid, and BAL fluid), is crucial for accurate and optimal results. Some general laboratory tests may provide clues of a fungal infection. Elevated serum lactate dehydrogenase (≥ 300 IU/mL) may serve as an adjunctive marker of PCP and histoplasmosis.^{38,39} An elevated aspartate aminotransferase to alanine aminotransferase ratio has been reported in disseminated histoplasmosis but not blastomycosis or coccidioidomycosis.⁴⁰ Peripheral BAL fluid and lung tissue eosinophilia may be seen in up to 50% of cases of coccidioidal pneumonia^{41,42}; eosinophilia is uncommon with all other fungal infections.

Conventional Testing

Microscopic Examination: Fungal morphology can be gleaned from microscopic assessment of clinical specimens using direct microbiologic stains, cytopathology, or histopathology. Evaluation of sputum, oral washes, BAL, and lung biopsy specimens are employed for the diagnosis of fungal pneumonia. The microscopic characteristics of visualized fungi can often provide a preliminary diagnosis of the infecting fungus and in some cases (such as visualization of characteristic *Coccidioides* spherules) a definite diagnosis. When unicellular yeasts are visualized, the size, presence, and type of budding (narrow vs broad-based), wall thickness, and the presence of capsules and of pseudohyphae can help narrow the differential diagnosis. For example, a medium-sized (8–40 μm) broad-based budding yeast

with thick double refractile walls is likely to be *Blastomyces*. Similarly, when hyphal structures are seen, hyphal width, pigmentation, consistency (ribbon-like vs firm), angle of branching, and presence of regular septations can provide a preliminary identification. Broad hyphae that are seldomly septated, branch at 90° angles, and twist in ribbon formations are most likely a Mucorales mold. Notably, genus and species-level identification of molds using microscopic evaluation alone is unreliable. Specialized stains are available to augment the diagnosis of certain pathogens (such as the Calcofluor white stain that stains chitin in fungal cell walls, Mucicarmine stain to detect the cryptococcal capsule, and direct and indirect fluorescence antibody stains for detection of *Pneumocystis*). At times, fungal elements are seen but not complete or characteristic enough to render a confident diagnosis (Fig 6). As with culture, negative microbiologic stains, cytopathology, or histopathology generally do not rule out fungal infection because these methods are insensitive.^{43,44}

Culture: Culture remains the gold standard for diagnosis of fungal pneumonia. In the right clinical scenario, growth of a fungus from a clinical specimen serves as compelling evidence of infection. When the clinical syndrome is not compatible, a positive fungal culture is reflective of airway colonization or, less commonly, specimen contamination. Fungal cultures also provide important antimicrobial sensitivity information for fungi with a potential for antifungal resistance such as *Candida* and *Aspergillus*. Various fungal pathogens grow at different rates, ranging from 1 to 3 days (*Candida*, *Cryptococcus*, Mucorales) to 3 to 7 days (*Aspergillus*, most hyaline and dematiaceous molds, *Coccidioides*) to 2 to 3 weeks (*Histoplasma*, *Blastomyces*, slow-growing dematiaceous molds).⁴⁵ This has implications for clinical diagnosis because other diagnostic modalities may be needed to diagnose slower-growing fungi more promptly. Although useful when positive, fungal cultures are generally very insensitive. For example, respiratory cultures are less than 30% sensitive for acute pulmonary aspergillosis, coccidioidomycosis, and histoplasmosis.^{46–48} *Pneumocystis* does not grow on culture. Therefore, negative cultures do not rule out the possibility of fungal pneumonia.

Antigen Testing: Detection of fungal cell wall antigens in blood, urine, and in some cases BAL specimens is a highly useful noninvasive and sometimes rapid method for the diagnosis of certain fungal pneumonias, including cryptococcosis, aspergillosis, pneumocystosis,

TABLE 2] Conventional Diagnostics and Diagnostic Approach for Specific Fungal Pneumonias

Fungal Pathogen	Suggested Diagnostic Tests
<i>Aspergillus</i> (invasive)	• Culture (gold standard, low sensitivity) • Microscopic examination (gold standard, low sensitivity) • BAL and serum galactomannan (in patients with prolonged neutropenia) • Serum and blood <i>Aspergillus</i> PCR (especially in areas with high prevalence of resistant <i>Aspergillus</i>) • Tissue-based <i>Aspergillus</i> PCR • Serum (1→3)-β-D-Glucan (in patients with prolonged neutropenia) Diagnostic approach: • Start with serum galactomannan and (1→3)-β-D-Glucan in patients with prolonged neutropenia and obtain sputum for fungal culture and microscopy. • Plan for bronchoscopy with BAL (for fungal cultures microscopy and cytology) and transbronchial biopsy vs interventional radiology-assisted transthoracic biopsy (for fungal cultures and histopathology), depending on lesion accessibility. • If available, <i>Aspergillus</i> PCR can be performed on blood or BAL. • If histopathology demonstrates compatible fungal hyphae but cultures are negative, <i>Aspergillus</i>-specific or fungal broad-range PCR testing of ITS PCR and 18S rDNA can be performed on tissue specimens. • Also consider the possibility of other sites of infection (especially brain, sinuses). • EORTC criteria can help with assessing the probability (possible, probable, proven) of infection.
Mucorales	• Culture (gold standard, low sensitivity) • Microscopic examination (gold standard, low sensitivity) • Serum mucorales PCR (if available) • Tissue-based mucorales PCR (in patients with hematologic malignancies or prolonged neutropenia) Diagnostic approach: • Obtain sputum for fungal culture and microscopy. • Serum mucorales PCR can be performed in patients with prolonged neutropenia if available. • Plan for urgent bronchoscopy with BAL (for fungal cultures microscopy and cytology) and transbronchial biopsy vs interventional radiology-assisted transthoracic biopsy (for fungal cultures and histopathology), depending on lesion accessibility. • If histopathology demonstrates compatible fungal hyphae but cultures are negative, <i>Mucorales</i>-specific or fungal broad-range PCR testing of ITS PCR and 18S rDNA can be performed on tissue specimens. • Consult thoracic surgery for possible surgical resection. • Also consider the possibility of other sites of infection (especially sinuses). • EORTC criteria can help with assessing the probability (possible, probable, proven) of infection.
<i>Pneumocystis jirovecii</i>	• Microscopic examination (gold standard, lower sensitivity) • Pneumocystis PCR on BAL and other specimens (oral wash, induced sputum, lung tissue) • Pneumocystis DFA/IFA on BAL • Serum (1→3)-β-D-Glucan Diagnostic approach: • Start with serum (1→3)-β-D-Glucan. • Plan for bronchoscopy with BAL ± transbronchial biopsy (for cytology and PCP PCR or PCP DFA/IFA on BAL). • If bronchoscopy cannot be performed, perform PCP PCR on oral wash or induced sputum. • EORTC criteria can help with assessing the probability (possible, probable, proven) of infection.
<i>Cryptococcus</i>	• Culture (gold standard, lower sensitivity) • Microscopic examination including Fontana-Mason and Mucicarmine stains (gold standard, low sensitivity) • Serum <i>Cryptococcus</i> antigen (high sensitivity) • Tissue-based <i>Cryptococcus</i> PCR Diagnostic approach: • Start with serum <i>Cryptococcus</i> antigen. If this is negative, pulmonary cryptococcosis is unlikely. • If testing is negative but suspicion remains high, especially if pulmonary nodules are present, plan for bronchoscopy with BAL (for fungal cultures microscopy and cytology) and transbronchial biopsy vs interventional radiology assisted transthoracic biopsy (for fungal cultures and histopathology), depending on lesion accessibility. • If histopathology demonstrates compatible yeast but cultures are negative, Fontana-Mason and Mucicarmine stains as well as <i>Cryptococcus</i>-specific or fungal broad-range PCR testing of ITS PCR and 18S rDNA can be performed on tissue specimens. • Also consider the possibility of other sites of infection (especially brain, skin, prostate). • EORTC criteria can help with assessing the probability (possible, probable, proven) of infection.

(Continued)

TABLE 2] (Continued)

Fungal Pathogen	Suggested Diagnostic Tests
<i>Histoplasma</i>	• Culture (gold standard, low sensitivity) • Microscopic examination (gold standard, low sensitivity) • <i>Histoplasma</i> antigen (urine, serum, BAL) • <i>Histoplasma</i> serologies (enzyme-linked immunoassay, complement fixation, immunodiffusion) • Serum (1→3)-β-D-glucan • Tissue-based <i>Histoplasma</i> PCR Diagnostic approach: • Start with urine <i>Histoplasma</i> antigen. • Consider obtaining a serum <i>Histoplasma</i> antigen and <i>Histoplasma</i> serologies as well (combination increases sensitivity). ◦ Serology can be falsely negative early in disease course and in immunosuppressed patients. • If testing is negative but suspicion remains high, especially if pulmonary nodules are present, plan for bronchoscopy with BAL (for fungal cultures microscopy and cytology) and transbronchial biopsy vs interventional radiology assisted transthoracic biopsy (for fungal cultures and histopathology), depending on lesion accessibility. • If histopathology demonstrates compatible yeast but cultures are negative, <i>Histoplasma</i>-specific or fungal broad-range PCR testing of ITS PCR and 18S rDNA can be performed on tissue specimens. • Also consider the possibility of other sites of infection (especially brain, skin). • EORTC criteria can help with assessing the probability (possible, probable, proven) of infection.
<i>Blastomyces</i>	• Culture (gold standard, low sensitivity) • Microscopic examination (gold standard, low sensitivity) • <i>Blastomyces</i> antigen (urine, serum, BAL) • <i>Blastomyces</i> serologies (low sensitivity and specificity) • Tissue-based <i>Blastomyces</i> PCR Diagnostic approach: • Start with urine <i>Blastomyces</i> antigen. • Serology is low sensitivity and specificity and not generally recommended. • If testing is negative but suspicion remains high, especially if pulmonary nodules are present, plan for bronchoscopy with BAL (for fungal cultures microscopy and cytology) and transbronchial biopsy vs interventional radiology assisted transthoracic biopsy (for fungal cultures and histopathology), depending on lesion accessibility. • If histopathology demonstrates compatible yeast but cultures are negative, <i>Blastomyces</i>-specific or fungal broad-range PCR testing of ITS PCR and 18S rDNA can be performed on tissue specimens. • Also consider the possibility of other sites of infection (especially brain, skin, bone, prostate). • EORTC criteria can help with assessing the probability (possible, probable, proven) of infection.
<i>Coccidioides</i>	• Culture (gold standard, low sensitivity) • Microscopic examination (gold standard, low sensitivity) • <i>Coccidioides</i> serologies (enzyme-linked immunoassays, complement fixation, immunodiffusion) • <i>Coccidioides</i> antigen (urine) • Serum (1→3)-β-D-glucan • Tissue-based <i>Coccidioides</i> PCR Diagnostic approach: • Start with <i>Coccidioides</i> serology (enzyme-linked immunoassay). • If positive, confirm with complement fixation or immunodiffusion to rule out false positive. • Serology can be falsely negative early in disease course. ◦ Consider repeating serology in 2-6 wk. • Serology can be falsely negative in immunocompromised patients. ◦ Consider sending urine <i>Coccidioides</i> antigen testing. • If testing is negative but suspicion remains high, especially if pulmonary nodules are present, plan for bronchoscopy with BAL (for fungal cultures microscopy and cytology) and transbronchial biopsy vs interventional radiology assisted transthoracic biopsy (for fungal cultures and histopathology), depending on lesion accessibility. • If histopathology demonstrates compatible yeast but cultures are negative, <i>Coccidioides</i>-specific or fungal broad-range PCR testing of ITS PCR and 18S rDNA can be performed on tissue specimens. • Also consider the possibility of other sites of infection (especially brain, nasal, skin). • EORTC criteria can help with assessing the probability (possible, probable, proven) of infection.

DFA = direct fluorescent antibody; EORTC = European Organisation for Research and Treatment of Cancer; IFA = indirect fluorescent antibody; ITS = internal transcribed spacer; PCP = *Pneumocystis jirovecii* pneumonia; PCR = polymerase chain reaction.

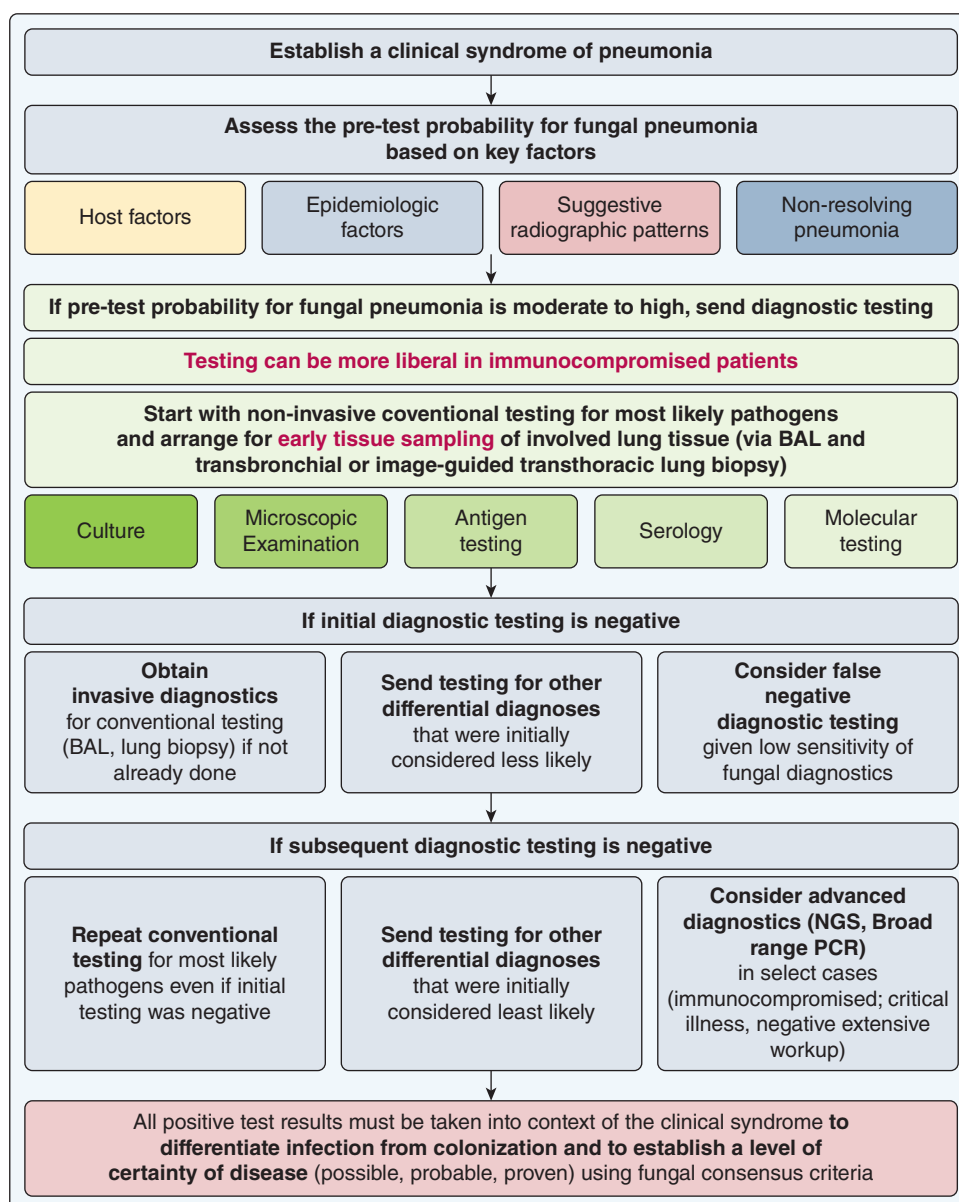


Figure 5 – A diagnostic framework for fungal pneumonia.

and dimorphic fungal infections. Some antigen tests are highly sensitive (cryptococcal antigen: 80%-100%) and others are less so (serum *Aspergillus* galactomannan antigen: 20%-90%).^{45,49} The (1→3)-β-D-Glucan assay is a pan-fungal antigen that can be detected in most fungal infections, with the exception of cryptococcosis, blastomycosis, and mucormycosis, in which it is low positive or negative. This assay is particularly useful for the noninvasive diagnosis of *Pneumocystis* pneumonia. The positive predictive value of an elevated (1→3)-β-D-Glucan in patients with appropriate clinical and radiographic features (usually a diffuse interstitial pneumonia) is greater than 90%, whereas the negative

predictive value is 95%.^{33,50} Of note, assay performance is higher in individuals with HIV. Combining (1→3)-β-D-Glucan with pneumocystis polymerase chain reaction (PCR) in BAL increases the specificity for PCP to 100%. The serum (1→3)-β-D-Glucan and *Aspergillus* galactomannan antigen are most sensitive in immunocompromised patients with prolonged neutropenia and should be used predominantly in this population because sensitivity is low or unknown in other populations.^{50,51} The sensitivity of antigen testing is generally higher in urine compared with serum, in BAL compared with urine or serum when available (*Aspergillus*, *Histoplasma*), and in highly

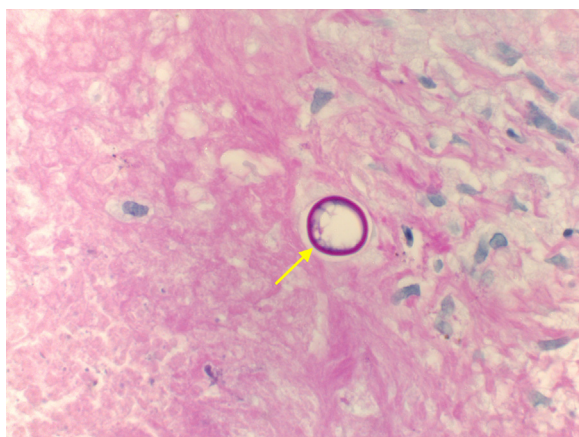


Figure 6 – Fungal elements on histopathology. At times, fungal elements seen on histopathology are insufficiently specific to render a confident diagnosis. This photomicrograph (periodic acid-Schiff stain) of a lung biopsy from a patient with nonresolving pneumonia reveals a large indeterminate yeast-like structure (yellow arrow). On close inspection, endosporeulation can be noted on the inner left portion of the structure, which is suggestive of *Coccidioides immitis/posadasii*. Nucleic acid amplification applied to the lung biopsy specimen confirmed coccidioidomycosis. (Original magnification, $\times 400$).

immunocompromised patients (especially those with prolonged neutropenia), who often have a higher burden of infection.^{45,52} When performed in local laboratories, antigen tests can provide results in a short, clinically actionable timeframe. False positive antigen testing may occur. (1 $\rightarrow$ 3)- β -D-Glucan assay may be falsely positive with use of gauze, blood products, albumin, IV immunoglobulins, or *Nocardia* infection, among other causes, whereas *Aspergillus* galactomannan may be false positive with use of electrolyte solutions and consumption of certain foods.⁴⁵ Cross-reactivity with beta-lactam antibiotics is no longer a common cause of false positives.⁵³ Antigen tests for dimorphic fungal infections cross-react significantly with other dimorphic infections; for example, the *Histoplasma* antigen test exhibits close to 100% cross-reactivity with blastomycosis.^{45,47}

Serology: The utility of antibody testing for fungal pneumonia is limited, with some exceptions. Serologic testing for *Coccidioides* is recommended for diagnosis of pulmonary coccidioidomycosis. Complement fixation *Coccidioides* antibody testing provides quantitative titers that are useful for diagnosis (even low titers of 1:2 are considered significant), assessment of disease severity (titers $\geq$ 1:16 are associated with disseminated disease), and treatment response. Cross-reactivity may occur with other dimorphic fungal infections. Serologic testing for *Histoplasma capsulatum* can aid in the diagnosis of acute and chronic pulmonary histoplasmosis, whereas

serologic testing for *Blastomyces* has low utility because of low sensitivity and specificity.⁴⁷ When the clinical picture is compatible and other diagnostics are negative, antibody testing for *Aspergillus* may assist with the diagnosis of chronic pulmonary aspergillosis and of allergic manifestations such as allergic bronchopulmonary aspergillosis by providing evidence of *Aspergillus* exposure. Serology is less sensitive in patients with mild disease and in immunocompromised patients, particularly those on B-cell depleting therapy and in the first 2 weeks after an infection.

Molecular Testing: Molecular testing plays an important role in the diagnosis of some fungal infections. *Pneumocystis jirovecii* PCR testing on respiratory specimens is highly sensitive for detection of the organism and can help with diagnosis of PCP. However, a positive *Pneumocystis* PCR result in the absence of appropriate clinical and radiographic context is indicative of colonization, not infection.⁴⁴ Combining *Pneumocystis* PCR with serum (1 $\rightarrow$ 3)- β -D-Glucan testing increases the specificity for PCP.⁵⁰ *Aspergillus* PCR assays on respiratory specimens and blood are sensitive and specific for the detection of invasive aspergillosis; some assays provide genotypic resistance data, which are useful in geographic areas with a high incidence of azole-resistant *Aspergillus*.⁵⁴ A research-based serum Mucorales quantitative PCR assay was 85.2% sensitive and 89.9% specific for diagnosis of proven or probable mucormycosis in patients with hematologic malignancies and has potential for use in real-world settings.⁵⁵ Laboratory-developed molecular assays for other fungi are available, but their performance characteristics are not well established.

Advanced Testing

Broad-range PCR testing, a method that targets conserved regions of fungal DNA such as 18s rRNA or internal transcribed spacer 1 and 2, can be applied to fresh or fixed tissue or body fluids. The sensitivity is highest when organisms are visualized on microscopic stains; thus, broad-range PCR is most useful when stains are positive for fungi but cultures and other diagnostics are negative.⁵⁶ Routine testing of clinical specimens by broad-range PCR adds little diagnostic value and is not cost-effective.⁵⁷ Next-generation sequencing (NGS) in which the nucleic acid in a clinical specimen is extracted followed by unbiased (universal primers) or targeted (specific primers) sequencing, carries significant potential for diagnosis of fungal pneumonia. As with broad-range PCR, NGS is most sensitive when fungi are

visualized on stains. However, the clinical utility of current NGS assays for fungal infections, including pneumonia, is very low, ranging from 0.5% to 3.7%.^{57,58} NGS should be considered for the diagnosis of invasive fungal pneumonia in immunocompromised patients when standard diagnostics are negative but suspicion remains high.⁵⁹ There are several technologies with the potential for diagnosis of fungal pneumonia in the research pipeline. One such assay is gas chromatography-mass spectrometry, which detects volatile organic compounds produced by the metabolism of infecting fungi in exhaled breath. This method was found to be highly sensitive and specific for the diagnosis of invasive pulmonary aspergillosis.⁶⁰

Pitfalls of Fungal Diagnostics

Even in ideal circumstances, the sensitivity of diagnostic tests for fungi is generally poor, so negative results do not exclude the possibility of fungal infection. As a result, the European Organisation for Research and Treatment of Cancer consensus criteria, which consider clinical, radiographic, host, and microbiological factors, have been developed to assist with diagnosis of invasive fungal infection at various levels of certainty (proven, probable, possible).⁴⁴ When initial diagnostics for fungal pathogens are unrevealing, additional diagnostic testing should be pursued, ideally through tissue sampling if not already obtained. Reasons for false negative tests should be contemplated, and advanced diagnostics (broad-range PCR, NGS) should be considered.

Conclusions

Although less common than bacterial pneumonia, the incidence of fungal pneumonia is increasing because of a growing at-risk population of immunocompromised individuals as well as anthropogenic global heating and environmental disruption. The patient in the case vignette was ultimately diagnosed with pulmonary coccidioidomycosis after a transbronchial biopsy of the largest lung nodule showed characteristic spherules on histopathology. Both serology and urine antigen testing for *Coccidioides* were negative, illustrating the low sensitivity of these modalities, the importance of tissue sampling, and the need to maintain a high index of suspicion despite initial negative testing when host, epidemiologic, clinical, and radiographic characteristics are suggestive of a fungal pneumonia.

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